UNIVERSITY OF PENNSYLVANIA

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WALTER S. ROOT

A THESIS

IN PHYSIOLOGY

PRESENTED TO THE FACULTY OF THE GRADUATE SCHOOL IN

PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR

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The inter-relationship between oxygen and carbon dioxide in supporting the respiration of higher animals is a matter of fundamental importance. Since experiments upon such forms are often difficult to interpret, because of the number of uncontrollable factors involved, single cells have been used in the present study in which the material employed was *Paramecium caudatum* and the fertilized eggs of *Arbacia punctulata.*

Few investigations have been made concerning the effect of carbon dioxide upon the oxygen consumption of protozoa or of marine eggs, though Warburg (1910) observed no change in the respiration of fertilized sea urchin eggs in the presence of a carbon dioxide tension of 15 mm. Hg, and Burfield (1928) reported that small amounts of carbon dioxide profoundly decreased the rate of oxygen consumption of plaice eggs. In these studies, however, oxygen was measured by the Winkler method which, while accurate for the determination of dissolved oxygen in solutions free of organic materials, is said to be untrustworthy for egg suspensions (Heilbrunn, 1915; Warburg, 1914*a*). Moreover, the presence of iron in sea urchin eggs (Warburg, 1914*b*) is known to introduce large errors into the method (Alsterberg, 1926). Previous work on the effect of carbon dioxide upon the rate of oxygen consumption of single cells appears, therefore, to be not entirely satisfactory and a reinvestigation of the question by other methods has seemed desirable. In the experiments here recorded, the technique employed was that developed by Novy and his collaborators (1925) for bacterial respiration, and modified by Amberson (1928) for the respiration of unicellular animal organisms.

Experiments with Paramecium

A thick suspension of the protozoa was obtained by placing several liters of culture in a large glass cylinder which was illuminated near

the top by rays of light from a sixty-watt electric light bulb. The organisms under these conditions swim to the top, and may be siphoned off in great numbers. The suspension so obtained was then centrifuged at two thousand revolutions a minute for ten to twenty seconds. The centrifuge was stopped suddenly to prevent the organisms from swimming from the bottom of the centrifuge tubes before the supernatant fluid could be decanted. The sediment of protozoa was washed in several changes of boiled, cooled, filtered pond water. The suspension in its final form must have been relatively free from bacteria, because control experiments with the cells absent showed no measurable oxygen consumption. The cultures were never pure, but *P. caudatum* always constituted at least 95 per cent of the protozoa present. The original culture of *Paramecium* was obtained from Dr. William Canovan of the Zoölogy Department of the University of Pennsylvania.

In order to obtain two suspensions containing approximately the same number of cells, a calibrated glass " mixer " was used. This consisted of a glass tube three-quarters of an inch in diameter, fitted at each end with a ground glass stopper. It was separated into two chambers of about twenty cc. capacity by a stopcock, the bore of which was of the same diameter as the tube. The suspension was poured into and out of the " mixer " eight to ten times with one stopper in place and with the stopcock open. After pouring the suspension in for the last time, the second stopper was inserted and the stopcock turned before the protozoa had an opportunity to change their distribution. The suspensions on each side of the stopcock were then poured into two calibrated cylindrical glass vessels of about the size and shape of Haldane gas collecting tubes. These tonometers were fitted with three-way stopcocks at both ends. Twenty-five cc. of boiled, cooled, filtered pond water were added to each tonometer.

Gas mixtures were made up in two twenty-one liter bottles so arranged that, when air or nitrogen was forced into one, the water contained therein passed into the second bottle, displacing the gas mixture which had previously been made up to approximately the desired percentage of oxygen and carbon dioxide. While the water contained in these bottles absorbed a certain amount of the gas mixtures above it, the gas tensions were determined from samples of the gas after it had passed through the tonometer, and immediately before the tonometer was closed. Any changes in gas tension occurring within the bottles did not, therefore, result in errors in the respiratory determinations.

At the beginning of an experiment, the gas mixture was slowly bubbled through one of the suspensions. Temperature equilibrium was

achieved by placing the tonometers in a water bath at the same temperature as that at which the respiration was to be measured (25.6° ± 0.3° C.). Every few seconds the passage of the gas was suspended and, with the stopcocks closed, the tonometer was gently rocked to hasten the attainment of equilibrium. After such a procedure, a drop of water was left in the capillary part of the tonometer which leads to the stopcock. When the tonometer was opened to the outside air, the drop was displaced inwards, because the pressure within the tonometer was less than atmospheric, due to the absorption of gases by the suspension. Passage of the gas mixture was continued until the drop was no longer displaced. A sample of the gas mixture, after it had passed through the tonometer, was now collected in a Bailey sampling bottle. Care was exercised to prevent the presence of a positive pressure within the tonometer. The stopcocks were then closed and the tonometers were placed in a second water bath. The tonometers were rotated upon their long axes sixty times a minute. No cellular destruction or abnormal behavior was observed. The same cultures were used every second or third day.

While the first suspension was undergoing the above treatment, the second suspension was equilibrated in the same water bath with air, by connecting one end of the tonometer with a compressed air inlet or a water pump. After approximately the same length of time as was required for the equilibration of the tonometer containing carbon dioxide, an air sample was secured, the stopcocks were closed, and the tonometer was placed in the rotator. The two tonometers rarely differed by more than five to ten minutes in their starting times. At the end of two to four hours, the tonometers were removed from the water bath and a sample of the contained gas was withdrawn in a Bailey collector, and set aside for later analysis. Respiratory exchanges were calculated for three-hour experiments.

The gas samples were analyzed in duplicate by means of a Haldane-Henderson analyzer with a nitrogen side tube, the principle being that employed in Bazett's modification (1928). The analyses were controlled by daily air analyses, and were accurate to 0.03 to 0.04 per cent.

Assuming the gaseous solubilities to be those given by the standard tables for pure water at this temperature, the total oxygen and carbon dioxide present at the beginning and at the end of the experiment in both air and water were calculated, the usual correction for barometer, water vapor, etc., being applied. The results of a typical experiment are as follows:

Sample Experiment

Tonometers	VI	V
Volume of tonometers	99.36 cc.	99.47 cc.
Volume of suspension	45.01 cc.	45.01 cc.
Gas analysis at beginning		
O_2	20.91%	19.29%
CO_2	0.04%	7.50%
N_2	79.05%	73.21%
Gas analysis at end (corrected for volume change)		
O_2	18.71%	17.22%
CO_2	0.88%	8.61%
N_2	79.05%	73.21%
Oxygen in air and water		
at beginning	11.626 cc.	10.746 cc.
at end	10.403 cc.	9.593 cc.
Oxygen consumption	1.223 cc.	1.153 cc.
Carbon dioxide in air and water		
at beginning	0.036 cc.	6.788 cc.
at end	0.793 cc.	7.789 cc.
Carbon dioxide production	0.757 cc.	1.001 cc.
Volumes corrected to dry values at 0° C. and 760 mm. Hg		
Oxygen consumption	1.081 cc.	1.019 cc.
Carbon dioxide production	0.669 cc.	0.885 cc.
Respiratory quotient	0.618	0.868
Oxygen tension in mm. Hg	153.4–137.2	141.5–126.3
Carbon dioxide tension in mm. Hg	0.3– 6.4	55.0–63.1

Ratio oxygen consumption $\frac{V}{VI} = 0.942$

Ratio carbon dioxide production $\frac{V}{VI} = 1.322$

The results obtained in forty-five experiments are given in Table I and Fig. 1. It will be noted that at a carbon dioxide tension of about 15 mm. Hg, the curve of the rate of oxygen consumption rises, reaching a maximum at approximately 40 mm. Hg and, crossing the line of the control rate of oxygen consumption at about 67 mm. Hg, falls away at the higher tensions.

Examination of the tonometers with a low powered binocular microscope showed that an exposure of three hours to a carbon dioxide tension of about 150 mm. Hg noticeably decreased the motility of the protozoa. At about 220 mm. Hg the cells became shorter and thicker and the nuclei became more clearly visible, standing out sharply from the rest of the protoplasm. When the animals were subjected to a tension of 250 mm. Hg for three hours, some were irreversibly affected by the gas.

In all experiments, the oxygen tension was kept above 62 mm. Hg. Amberson (1928) has shown that the rate of oxygen consumption of *Paramecium* is constant between 200 and 50 mm. Hg partial pressure, so it is unlikely that the results here reported were due to a lack of

TABLE I

Respiration of Paramecium at Different Carbon Dioxide Tensions

Experiment No.	CO_2 Tensions in Tonometer B	Respiration in Tonometer A			Respiration in Tonometer B			Ratio O_2 Consumption $\frac{B}{A}$	Ratio CO_2 Production $\frac{B}{A}$
		O_2 Consumed	CO_2 Produced	*RQ*	O_2 Consumed	CO_2 Produced	*RQ*		
	mm. Hg	*cc.*	*cc.*		*cc.*	*cc.*			
1	0.4– 3.6	0.381	0.256	0.669	0.357	0.254	0.711	0.936	0.999
2	0.3– 12.1	1.159	0.871	0.751	1.116	0.924	0.827	0.962	1.060
3	0.4– 7.4	1.169	0.731	0.625	1.154	0.692	0.599	0.987	0.946
4	1.0– 8.1	1.264	0.647	0.511	1.302	0.732	0.562	0.970	1.131
5	16.7– 26.4	1.305	0.848	0.650	1.315	1.042	0.780	1.007	1.228
6	25.8– 33.9	0.776	0.535	0.691	0.817	0.895	1.096	1.052	1.672
7	29.1– 39.1	1.232	0.813	0.659	1.359	1.083	0.796	1.103	1.332
8	34.4– 37.4	0.410	0.288	0.702	0.452	0.347	0.729	1.102	1.204
9	39.3– 43.1	0.592	0.266	0.450	0.652	0.315	0.484	1.101	1.184
10	42.7– 50.3	0.813	0.474	0.613	0.907	0.713	0.823	1.115	1.504
11	43.0– 52.3	1.565	0.810	0.525	1.628	1.004	0.617	1.040	1.239
12	44.8– 54.2	0.813	0.511	0.617	0.886	1.012	1.144	1.067	1.980
13	48.0– 49.8	0.572	0.296	0.519	0.574	0.181	0.318	1.004	0.611
14	50.9– 58.2	1.314	0.789	0.601	1.485	0.779	0.525	1.130	0.987
15	52.4– 61.4	1.235	0.776	0.619	1.312	0.977	0.740	1.047	1.259
16	62.5– 69.5	1.117	0.599	0.536	1.187	0.763	0.642	1.062	1.273
17	69.3– 75.3	1.622	0.923	0.569	1.504	0.651	0.432	0.927	0.705
18	69.8– 79.0	1.674	0.925	0.552	1.573	0.889	0.565	0.939	0.961
19	80.5– 90.9	2.370	1.119	0.472	2.118	1.142	0.539	0.893	1.020
20	90.6– 97.3	0.774	0.554	0.716	0.644	0.662	1.027	0.832	1.176
21	91.4–110.8	3.260	1.859	0.570	3.021	2.018	0.668	0.926	1.085
22	94.7–100.9	0.864	0.643	0.744	0.810	0.589	0.727	0.937	0.916
23	102.6–118.8	2.417	1.448	0.599	2.222	1.689	0.760	0.919	1.166
24	105.6–127.4	3.839	2.152	0.565	3.495	2.334	0.667	0.910	1.089
25	126.7–135.3	0.863	0.565	0.654	0.736	0.814	1.105	0.852	1.440
26	140.9–149.9	1.197	0.812	0.678	0.950	0.964	1.014	0.793	1.187
27	141.6–151.4	0.872	0.645	0.739	0.594	1.071	1.803	0.681	1.660
28	155.0–164.8	1.228	0.789	0.642	0.927	1.004	1.083	0.754	1.272
29	158.2–166.2	1.710	1.015	0.593	1.333	1.213	0.909	0.779	1.195
30	160.8–171.2	1.246	0.888	0.712	0.791	1.027	1.298	0.634	1.156
31	167.3–173.7	0.827	0.514	0.621	0.534	0.473	0.885	0.645	0.920
32	173.8–185.3	2.196	1.308	0.595	1.494	1.334	0.893	0.680	1.019
33	181.8–192.8	1.293	0.997	0.771	0.892	0.867	0.972	0.689	0.869
34	202.2–204.1	0.970	0.667	0.687	0.506	0.184	0.363	0.521	0.275
35	206.8–210.8	1.499	0.946	0.631	0.702	0.395	0.562	0.469	0.418
36	217.4–222.0	2.032	1.124	0.553	1.004	0.525	0.521	0.494	0.467
37	224.7–229.4	0.828	0.488	0.589	0.511	0.502	0.982	0.617	1.028
38	224.9–233.5	0.918	0.509	0.554	0.317	0.914	2.812	0.345	1.795
39	234.5–241.2	0.610	0.385	0.631	0.360	0.713	1.980	0.590	1.851
40	238.2–246.8	0.990	0.673	0.679	0.504	0.713	1.414	0.509	1.059
41	253.3–257.1	1.296	0.679	0.524	0.556	0.385	0.692	0.429	0.567
42	270.2–276.9	1.177	0.787	0.629	0.454	0.693	1.526	0.385	0.880
43	277.2–296.7	1.606	1.010	0.628	0.852	1.711	2.008	0.530	1.694
44	353.9–361.0	1.558	1.017	0.652	0.843	0.715	0.848	0.541	0.703
45	418.1–423.8	0.860	0.398	0.462	0.288	0.599	2.079	0.334	1.505

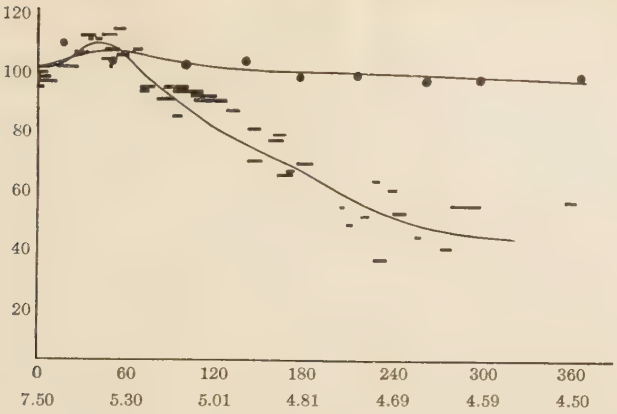

FIG. 1. The effect of carbon dioxide upon the oxygen consumption of *Paramecium*.

Abscissa: upper row of figures = mm. Hg carbon dioxide tension, lower row of figures = pH.

Ordinate = $\frac{\text{oxygen consumption in carbon dioxide tonometer}}{\text{oxygen consumption in control or air tonometer}}$.

— = carbon dioxide experiments.

● = hydrochloric acid experiments.

oxygen. It is, of course, possible that the oxygen tension at which the effects of oxygen deficiency appear, may not be the same in the presence of high tensions of carbon dioxide as in the relative absence of this gas. If the above experiments had been carried out in the region of oxygen deficiency, however, even slight increases in the oxygen tension would have resulted in an increased rate of oxygen consumption. That this was not the case is shown by a comparison of experiments 18 and 19 (Table II) which show that when the oxygen tension in tonometer *B* (CO_2) in experiment 18 was 62 per cent greater than that in tonometer *B* (CO_2), experiment 19, the differences in the rates of oxygen consumption lay within the error of the method. The carbon dioxide tensions in the two tonometers B (CO_2) do not differ by enough to affect appreciably the rates of the oxygen consumption in the two experiments.

TABLE II

Experiment No.	CO_2 tension	Tonometer A O_2 tension	O_2 Consumed	CO_2 tension	Tonometer B O_2 tension	O_2 Consumed	Ratio O_2 Consumption B/A
	mm. Hg	*mm. Hg*		*mm. Hg*	*mm. Hg*		
18	0.30– 9.20	156.5–130.3	1.758	69.8–79.0	142.27–115.8	1.504	0.939
19	0.30–10.64	156.8–121.4	2.370	80.5–90.9	93.8 – 62.4	1.774	0.893

The average of the respiratory quotients obtained in air in forty-eight determinations was 0.62. This is somewhat lower than the average value 0.69 obtained by Amberson (1928) in fourteen experiments. An examination of the respiratory quotients in the presence of different carbon dioxide tensions (Table I) shows that these values fluctuate irregularly between 0.32 and 2.81. When the forty-two experiments, carried out at different carbon dioxide tensions, and their controls are arranged in seven groups of six experiments each, and the respiratory quotients, and ratios of oxygen consumption and carbon dioxide production averaged, Table III is obtained. It will be seen that the rate of carbon dioxide production in the presence of carbon dioxide does not decrease as does the rate of oxygen consumption, but remains relatively constant. As a consequence, the respiratory quotients rise progressively as the carbon dioxide tension is increased.

TABLE III

Summary of Experiments in Table I (Paramecium caudatum)

Group	Experiments	CO_2 tension mm. Hg	*RQ* control	*RQ* CO_2 tonometer	Ratio O_2 Consumption $\frac{CO_2 \text{ tonometer}}{\text{control}}$	Ratio CO_2 Production $\frac{CO_2 \text{ tonometer}}{\text{control}}$
I.....	4–9	1.0– 43.1	0.610	0.741	1.056	1.289
II....	10–15	42.7– 61.4	0.582	0.694	1.067	1.096
III...	16–21	62.0–110.8	0.569	0.645	0.929	1.036
IV...	22–27	91.4–151.4	0.663	1.012	0.848	1.242
V....	28–33	155.0–192.0	0.655	1.006	0.696	1.072
VI...	34–39	202.0–246.0	0.574	1.205	0.506	0.972
VII..	40–45	238.0–423.0	0.595	1.428	0.454	1.068

It has been clearly shown that carbon dioxide enters cells with ease (Jacobs 1920*a*, 1920*b*, 1924), whereas hydrochloric acid penetrates cells very slowly, if at all (Loeb, 1909, Jacobs 1924). In order to determine whether carbon dioxide decreases the rate of oxygen consumption by some internal action on the cell, or merely by increasing the acidity of the external solution, a group of nine experiments was performed to determine the effects upon the respiration of *Paramecium* of approximately the same degrees of acidity produced by hydrochloric acid.

Boiled, filtered, cooled, pond water was equilibrated at different carbon dioxide tensions, and the hydrogen ion concentrations determined electrometrically, using a closed quinhydrone electrode (Fig. 2). The pond water, though poorly buffered, contained bicarbonate in a concentration approximately 0.00014 M. This was sufficient to give relatively stable potentials. The pH readings were accurate to 0.04 of a pH unit as shown by determinations made upon known solutions and by comparing a series of readings.

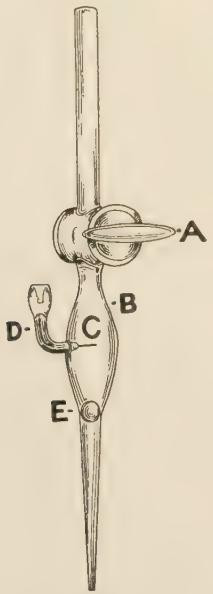

FIG. 2. Closed quinhydrone electrode.

To obtain hydrogen ion concentrations by means of hydrochloric acid, corresponding to those produced by carbon dioxide, pond water was made bicarbonate free by adding concentrated hydrochloric acid to bring it to a pH of 3 to 4, and aerated over night. It was then returned to the desired pH by the addition of concentrated sodium hydroxide.

The calculations of oxygen consumption and carbon dioxide production at different pH values appear in Table IV and Fig. 1. These results indicate that hydrochloric acid has no effect upon the respiration of *P. caudatum* at pH values as low as 4.5. The protozoa appeared in no way injured by these experiments.

TABLE IV

The Effect of Hydrochloric Acid on the Respiration of Paramecium

Experiment No.	Tonometer B pH	Resp. in Tonometer A			Resp. in Tonometer B			Ratio O_2 Consumption $\frac{B}{A}$
		O_2 Consumed	CO_2 Produced	*RQ*	O_2 Consumed	CO_2 Produced	*RQ*	
		cc.	*cc.*		*cc.*	*cc.*		
1	5.85	0.752	0.626	0.832	0.813	0.606	0.745	1.081
2	5.40	0.863	0.526	0.609	0.887	0.549	0.618	1.026
3	5.07	1.113	0.673	0.604	1.119	0.628	0.561	1.005
4	4.90	0.970	0.473	0.487	0.992	0.513	0.517	1.022
5	4.80	1.202	0.830	0.690	1.157	0.875	0.756	0.962
6	4.71	0.805	0.614	0.762	0.783	0.617	0.786	0.972
7	4.61	1.229	0.868	0.706	1.178	0.928	0.787	0.958
8	4.55	0.977	0.561	0.574	0.942	0.659	0.699	0.964
9	4.50	0.618	0.466	0.754	0.610	0.540	0.885	0.987

EXPERIMENTS ON FERTILIZED ARBACIA EGGS

Since it was impossible to determine how much of the depression in the rate of oxygen consumption of *Paramecium* was due to a suppression of the oxidative mechanism, and how much was dependent upon the decreased motility of the organism, it was necessary to perform similar experiments upon a non-motile cell. Experiments were, therefore, carried out on the fertilized eggs of *Arbacia punctulata* at Woods Hole.

The eggs were removed from the female, freed of ovarian debris by straining through cheese cloth, and washed in several changes of sea water. A heavy suspension of cells was secured by allowing the eggs to sediment in several finger bowls, and decanting the supernatant sea water. Eggs from twelve to fifteen females were used in each experiment. After fertilization, the excess spermatozoa were removed by allowing the cells to sediment in several changes of sea water. The suspension was then divided into two parts by means of the "mixer" used in the experiments with *Paramecium*. The two suspensions were equal to within 10 per cent as measured by the respiratory exchange in control experiments. The tonometers used in the experiments on *Paramecium* were also employed in these experiments.

At times varying from twenty to sixty minutes after fertilization, the suspensions were equilibrated simultaneously with air and with different carbon dioxide tensions in the same manner as described for *Paramecium*. At the conclusion of the equilibration in the water bath, the two tonometers were closed in such a manner that the contained gas was left at atmospheric pressure and at a temperature of 20.6° ±

0.3° C. Initial gas samples were secured in Bailey bottles as in the experiments with *Paramecium*. The tubes were then rotated on their long axes thirty to sixty times a minute at the equilibration temperature. Under these conditions the eggs were evenly distributed throughout the suspension. Cleavage proceeded in the normal manner, but it was somewhat delayed in time. A quantitative study of the retardation in cleavage at different carbon dioxide tensions will be published elsewhere by Haywood and Root (1930). Eighty to one hundred per cent cleavage was obtained in all the air tonometers except those indicated in Table V. Cytolysis during the experiments was not observed when fertilized eggs were used, although unfertilized eggs were found to be extremely fragile.

TABLE V

Respiration of Fertilized Arbacia Eggs at Different Carbon Dioxide Tensions

Experiment No.	CO_2 Tensions in Tonometer B	Respiration in Tonometer A			Respiration in Tonometer B			Ratio O_2 Consumption $\frac{B}{A}$	Ratio CO_2 Production $\frac{B}{A}$
		O_2 Consumed	CO_2 Produced	*RQ*	O_2 Consumed	CO_2 Produced	*RQ*		
	mm. Hg	*cc.*	*cc.*		*cc.*	*cc.*			
1	0.38– 2.92	0.722	0.346	0.479	0.664	0.318	0.486	0.920	0.919
2	0.30– 7.85	1.069	0.803	0.751	1.005	0.848	0.843	0.940	1.056
3	0.30– 5.03	0.816	0.504	0.619	0.762	0.550	0.722	0.934	1.091
4*	1.27– 10.07	1.285	0.963	0.749	1.161	0.844	0.726	0.903	0.876
5	1.55– 6.55	0.471	0.401	0.851	0.446	0.365	0.817	0.946	0.910
6	6.72– 10.82	1.031	0.734	0.711	0.823	0.436	0.529	0.798	0.594
7	6.97– 14.24	1.319	0.924	0.700	1.022	1.086	1.062	0.774	1.175
8	9.74– 19.77	2.406	1.564	0.650	1.755	1.178	0.671	0.722	0.753
9	12.58– 14.72	0.456	0.335	0.734	0.320	0.228	0.712	0.702	0.680
10*	16.23– 21.51	1.823	1.380	0.757	0.964	0.559	0.579	0.529	0.405
11	19.96– 25.19	1.794	1.163	0.648	0.993	0.603	0.607	0.553	0.518
12	25.40– 27.03	0.506	0.345	0.681	0.220	0.172	0.786	0.434	0.498
13	27.58– 29.28	1.154	0.848	0.734	0.426	0.175	0.410	0.369	0.206
14	34.67– 37.88	1.013	0.646	0.637	0.295	0.345	1.169	0.291	0.534
15	37.80– 40.68	1.103	0.910	0.804	0.324	0.306	0.928	0.293	0.336
16	42.33– 44.57	0.925	0.683	0.738	0.206	0.232	1.126	0.222	0.339
17	43.80– 45.07	0.608	0.359	0.590	0.173	0.131	0.754	0.284	0.365
18	51.73– 52.48	0.629	0.575	0.914	0.144	0.081	0.562	0.229	0.140
19	58.43– 60.16	1.111	0.761	0.685	0.205	0.181	0.882	0.184	0.236
20	63.93– 65.14	0.999	0.829	0.829	0.339	0.131	0.386	0.339	0.157
21	64.59– 65.49	0.883	0.629	0.712	0.187	0.101	0.540	0.211	0.160
22	71.51– 72.56	0.860	0.588	0.683	0.197	0.112	0.577	0.229	0.190
23*	85.50– 87.94	0.992	0.729	0.734	0.343	0.192	0.560	0.345	0.263
24*	86.90– 88.02	0.615	0.439	0.713	0.178	0.119	0.669	0.289	0.271
25	124.99–125.75	0.558	0.356	0.638	0.185	0.075	0.405	0.331	0.210
26	131.61–133.33	0.793	0.570	0.718	0.143	0.184	1.286	0.180	0.322
27	139.79–140.41	0.543	0.387	0.712	0.147	0.065	0.442	0.270	0.168
28*	162.92–163.15	0.972	0.775	0.797	0.176	0.024	0.136	0.181	0.030
29	176.02–177.19	0.558	0.413	0.740	0.045	0.125	0.277	0.081	0.302

* Less than 80 per cent cleavage in air tubes.

In the experiments whose duration deviated from three hours, the respiratory exchange was calculated for this time. At 20° C., the first cell division occurred about one hour after fertilization in the control tonometers. At the end of two hours the cells were in the four and eight cell stages; at the end of three hours, they were in the sixteen and thirty-two cell stages. The material was not, therefore, unicellular throughout the whole experiment. No differences were observed in the effects of carbon dioxide upon the rate of oxygen consumption in experiments in which the egg had started to cleave before introduction into the tonometers, as compared with eggs which had not started to cleave at that time.

At the end of the experiment, samples of gas were secured from both tonometers and analyzed. The amounts of oxygen and of carbon dioxide in the gas and in the sea water were then calculated for the beginning and for the end of the experiments. For this calculation, the absorption coefficients for oxygen and carbon dioxide in sea water given by Krümmel (1907) were used.

The results obtained in twenty-nine experiments are given in Table V and shown graphically in Fig. 3. It will be observed in Fig. 3

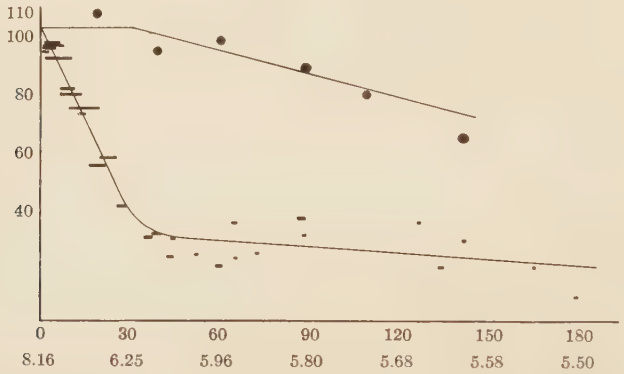

Fig. 3. The effect of carbon dioxide upon the oxygen consumption of fertilized *Arbacia* eggs.
Abscissa and ordinate as in Fig. 1.

— = carbon dioxide experiments.
● = hydrochloric acid experiments.

that, for each 10 mm. increase in the carbon dioxide tension up to 30 mm. Hg, there is a 21 per cent decrease in the rate of oxygen consumption. Above 30 mm. Hg, further increases in the carbon dioxide tension have a relatively slight effect upon the rate of oxygen consumption.

The average of the respiratory quotients obtained in air in thirty-two determinations was 0.71, which is lower than the average value of 0.78 obtained by Amberson (1928) in twenty experiments. The respiratory quotients in the presence of carbon dioxide must be interpreted with caution, for they range irregularly between 0.14 and 1.29. When the twenty-five carbon dioxide experiments in Table V are arranged in five groups of five experiments each, and the respiratory quotients and ratios of oxygen consumption and carbon dioxide production averaged, Table VI is obtained. It may be observed that, in the presence of carbon dioxide, the respiratory quotients are slightly higher than the control values up to 60 mm. Hg carbon dioxide tension. Above this tension, the respiratory quotients are lower. The carbon dioxide production and the oxygen consumption both decrease as the carbon dioxide tension increases.

TABLE VI

Summary of Experiments in Table V (Fertilized Arbacia Eggs)

Group	Experiments	CO_2 Tension mm. Hg	*RQ* Control	*RQ* CO_2 Tonometer	Ratio O_2 Consumption $\frac{CO_2 \text{ tonometer}}{\text{control}}$	Ratio CO_2 Production $\frac{CO_2 \text{ tonometer}}{\text{control}}$
I.....	5–9	1.6– 19.8	0.729	0.758	0.788	0.822
II....	10–14	16.2– 37.9	0.691	0.710	0.435	0.432
III...	15–19	37.8– 60.2	0.746	0.850	0.242	0.283
IV...	20–24	63.9– 88.0	0.734	0.546	0.282	0.208
V....	25–29	125.0–177.2	0.721	0.509	0.208	0.206

In order to determine whether carbon dioxide decreased the rate of oxygen consumption of fertilized *Arbacia* eggs by some internal effect, or merely by changing the pH of the sea water, a group of experiments was carried out in which approximately the same pH range was produced with hydrochloric acid which enters cells with difficulty, if at all. In these experiments, sea water was first made bicarbonate-free by adding concentrated hydrochloric acid to bring it to a pH of 3 to 4, and was ærated overnight. It was then returned to the desired pH by the addition of concentrated sodium hydroxide. The hydrogen ion concentration was determined by means of a quinhydrone electrode with an accuracy of 0.02 to 0.03 pH. The pH so obtained was compared with that of sea water at different carbon dioxide tensions as determined by Henderson and Cohn (1916).

The results of these experiments show a noticeable, but much smaller depression of the rate of oxygen consumption beginning at about pH 6.2 (corresponding to 35 mm. Hg carbon dioxide tension), and falling to about 62 per cent of the control at pH 5.6 (corresponding to that of sea water in equilibrium with carbon dioxide at 140 mm. Hg). (Table VII and Fig. 3.)

TABLE VII

The Effect of Hydrochloric Acid on the Respiration of Fertilized Arbacia Eggs

Experiment No.	Tonometer B pH	Resp. in Tonometer A			Resp. in Tonometer B			Ratio O_2 Consumption $\frac{B}{A}$
		O_2 Consumed	CO_2 Produced	*RQ*	O_2 Consumed	CO_2 Produced	*RQ*	
		cc.	*cc.*		*cc.*	*cc.*		
1	6.43	0.670	0.302	0.450	0.702	0.289	0.410	1.048
2	6.15	0.598	0.282	0.471	0.550	0.302	0.549	0.919
3	5.96	0.661	—	—	0.630	0.200	0.317	0.953
4	5.81	0.473	0.255	0.539	0.405	0.237	0.585	0.856
5	5.71	0.541	0.054	0.099	0.417	0.220	0.527	0.770
6	5.60	0.448	0.207	0.462	0.278	0.172	0.618	0.620

DISCUSSION

While the increased rate of oxygen consumption of *Paramecium* between carbon dioxide tensions of 15 and 66 mm. Hg probably lies within the experimental error, no experiment within this range resulted in a decreased rate of oxygen consumption. Since it is generally known that carbon dioxide causes an initial increase in the motility of *Paramecium* and other ciliates, followed by a decrease, it is suggested that the increased rate of oxygen consumption observed is a result of the increased motility of the organisms induced by carbon dioxide. This view is strengthened by the experiments performed upon *Arbacia* eggs in which there was no independent motility, and in which no increase in the rate of oxygen consumption was observed at any carbon dioxide tension.

Hydrochloric acid exerts a far less profound effect upon the rate of oxygen consumption of *Paramecium* and the fertilized eggs of *Arbacia* than does carbon dioxide at the same pH. Jacobs (1920*a*, 1920*b*, 1924) has shown that carbon dioxide far surpasses the acids which he studied in the rapidity with which its effects are produced. Hydrochloric acid, on the other hand, penetrates cells very slowly, if at all (Loeb, 1909; Jacobs, 1924). The differences in the results obtained by the action of carbon dioxide and of hydrochloric acid upon

the rate of oxygen consumption of fertilized *Arbacia* eggs and *Paramecium* may perhaps be interpreted as due to differences in the ability of the two acids to penetrate the cell membrane, and to produce within the cell changes in pH, though it is, of course, possible that the relatively profound effect of carbon dioxide upon the rate of oxygen consumption of these cells may be due to some other specific effect of its molecule.

The respiratory quotients of *Paramecium* rise progressively as the carbon dioxide tension is increased (Table III). It is possible that the suppression of oxidations under these conditions results in the production of acid metabolites which drive out carbon dioxide from bicarbonate contained in the cells and in the surrounding medium. The respiratory quotients of the sea urchin eggs, however, do not increase in the presence of high carbon dioxide tensions (Table VI). In these cells it is possible that acid substances are not produced when the rate of oxygen consumption is decreased by carbon dioxide, or that such acid substances may be rapidly reconverted into a non-acid form so that they do not accumulate in appreciable amounts. It would appear, assuming the absence of gross errors, that the respiratory reactions of the two cells to carbon dioxide differ.

I wish to express my appreciation for the interest and stimulating suggestions made by Dr. W. R. Amberson, under whose direction these experiments were performed.

Summary

1. The respiratory exchanges of *Paramecium caudatum* and the fertilized eggs of *Arbacia punctulata* have been studied by the method of gas analysis.

2. When *Paramecium* is exposed to different gas mixtures containing carbon dioxide at progressively increasing tensions, the rate of oxygen consumption increases slightly at the lower tensions and decreases at the higher tensions. The rate of oxygen consumption of fertilized *Arbacia* eggs is depressed by carbon dioxide at all of the tensions studied.

3. Moderate changes in the pH of the surrounding medium produced by hydrochloric acid have no apparent effect upon the rate of oxygen consumption of *Paramecium*. Similar changes in pH produced by hydrochloric acid diminish the rate of oxygen consumption of fertilized *Arbacia* eggs, although less markedly than does carbon dioxide at the same hydrogen ion concentration. The greater effectiveness of carbon dioxide in producing changes in the respiratory rate is presumably related to its greater penetrating power.

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